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WATER MONITORING MANAGEMENT

James Mumman, Administrator

October/November 1996

**SEDIMENT TOXICITY TEST
USING THE AMPHIPOD**

Hyaella azteca

(Furnace Brook, Beaver Brook)

October/November 1996

Assay Number(s): 96H007a, 96H007b, 96H007c

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EXECUTIVE SUMMARY

A toxicity test using the amphipod *Hyaella azteca* was performed on sediments collected from Furnace Brook (AN0042) and a reference site on Beaver Brook (AN0045) in the Delaware Basin. The reference was selected on Beaver Brook due to a "non-impaired" biological assessment by the Ambient Biomonitoring Network (AMNET). The Furnace Brook site was chosen because it was suspected of toxicity due to a "severely impaired" assessment by the AMNET program. The reference site on Beaver Brook exhibited a high percentage of mortalities, likely due to a predator organism found in the test vessels at the end of the test. Therefore, the test site was not compared to the reference, but to the control. The test site did not exhibit acute toxicity, in survival results, when statistically compared to the control. Further routine AMNET bioassessments will determine if additional testing is necessary.

INTRODUCTION

The Ambient Biomonitoring Network (AMNET) program is designed to establish biologically impaired stream segments throughout the state using EPA's Rapid Bioassessment Protocol (RBP). The RBP assesses impairment through the collection, identification, and classification of macroinvertebrates. Although the RBP is an excellent way in which to assess impairment, it may sometimes be difficult to distinguish if impairment is due to water quality or habitat destruction. Sediment Toxicity Testing is an additional tool to narrow down the cause of impairment to an acute toxicity problem before resorting to costly chemical monitoring.

Hyalella azteca is an epibenthic detritovore reported to also digest bacteria and algae from ingested sediment particles (Hargrave, 1970). This amphipod burrows into the sediment surface and inhabits lakes, ponds, and streams throughout North and South America (de March, 1981; Pennak, 1989). *H. azteca* is a sensitive benchmark species that can be cultured in the laboratory with relative ease.

METHODS

Sample sites were selected based on available AMNET data (see appendix a) and proximity to NJPDES facilities.

The sites selected are as follows (see map):

<u>AMNET STATION#</u>	<u>BIOLOGICAL ASSESSMENT</u>	<u>LOCATION</u> (see map)
AN0042	severely impaired	Furnace Brook @ Pequest Rd., Oxford
AN0045	non-impaired	Beaver Brook @ upstream of Silver Lake, Hope Twp.

Sediment samples were collected from sites AN0042 and AN0045 on October 16, 1996 at 10:45 and 11:20 hours respectively. At each station the sediment was collected in the stream channel using a stainless steel scoop sampler and placed into one liter amber glass bottles and stored at less than 4EC until the start of the test (NJDEP, 1992).

Prior to test initiation the sample sites were assigned assay numbers as follows:

96H007a = control
96H007b = AN0042
96H007c = AN0045

Testing methodology followed the Bureau of Water Monitoring Standard Operating Procedures (NJDEP, SM001.0795, 1995). 24 hours prior to the start of the test, the sediment from each station was mixed to provide a homogeneous sample and hand picked of any visible indigenous organism. For each site, 100 ml of sediment was added to each of the five 300 ml replicate test vessels and topped with laboratory grade freshwater to the 250 ml mark. The test vessels were then held at the test temperature (23EC) for 24 hours to allow the sediment to settle(NJDEP, SM001.0795,1995). After this time period, the overlying water was syphoned, and fresh water was added. A control set of replicates was also set up using 250 ml of overlying water only.

1 - 7 day *H. azteca* juveniles were collected and held for one week prior to the start of the test (NJDEP, 1995).

The test was initiated on October 22, 1996 at 10:45 hours, by adding ten 7 - 14 day old organisms from the holding chamber to each test series replicate. Each day the overlying water was exchanged, and each test replicate was fed 1.5 ml of YCT and 1.5 ml of the green algae *Selenastrum capricornutum* at a concentration of 35×10^6 cells/ml. Mortalities were noted if visible. pH, dissolved oxygen, and conductivity were measured from aliquots of each test series; measurements were made at the start of the test and after each 24 hour period (see table 3).

The test was concluded after ten days (November 1, 1996). Live organisms were counted (see table 1) and the dry weights measured (see table 2). Statistical analysis was performed, following EPA guidelines (U.S.E.P.A., 1991). The reference test was compared against the control and the remaining tests compared to the reference, providing the reference and the control were statistically the same.

RESULTS

The test was valid by meeting the acceptability requirements of $\geq 80\%$ survival (see table 1) in the control test series (NJDEP, SM001.0795,1995). The survival data was not normally distributed when analyzed by the Shapiro-Wilks test for normality, and therefore the Wilcoxon Rank Sum Test was used when comparing test survival results. There was a significant difference between the reference test, 96H007b, survival results and the control survival results. The reference test was not used to compare with the test site. Test 96H007b was then compared to the control. The survival data was not normally distributed when analyzed by the Shapiro-Wilks test for normality, and therefore the Wilcoxon Rank Sum Test was used when comparing test survival results. 96H007b showed no significant difference from the control for mortality. (see appendix b for statistical printout)

On days 4, 9, and 10, the Dissolved Oxygen fell below 40% saturation in 96H007b. The D.O. was 35%, 31%, and 38% respectively.

All visible indigenous organisms were removed from the sediment samples. However, a predatory nemertea (proboscis worm), *Prostoma rubrum*, was discovered in the vessels of the reference test.

DISCUSSION

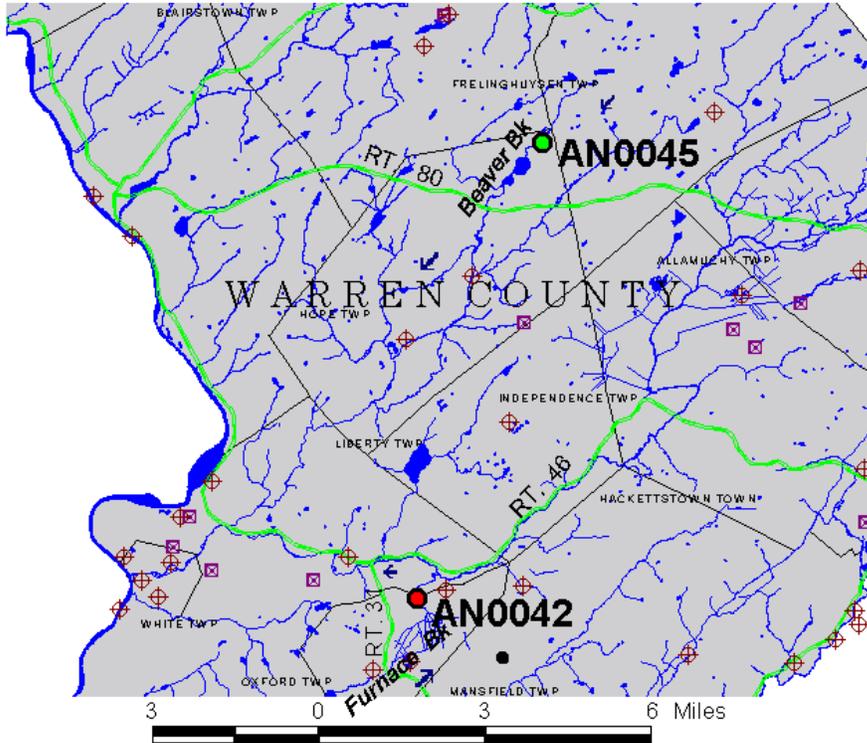
The sample site on Furnace Brook was chosen based on the results of macroinvertebrate studies and the proximity of NJPDES facilities and urbanization. Site AN0042 had severely impaired bioassessment results as analyzed in AMNET. The reference site, AN0045, was chosen because it had a nonimpaired bioassessment based on results from the AMNET program and was within the same major drainage basin as the test site. Similar stream morphology and similar ecological region designation to the sample site suspected of toxicity also factored into choosing the reference site.

The reference test showed a significant number of mortalities and was not used to compare with the Furnace Brook site. Beaver Brook is a highly productive stream with a large macroinvertebrate community. All visible indigenous organisms were removed from the sediment before the test was initiated. However, at the end of the test, a predatory nemertea, *Prostoma rubrum*, was discovered in the test vessels. Since as many as possible indigenous organisms were removed from the sample, this predator would only have the test organisms available as prey. If prey is large, such as *H. azteca*, *Prostoma* stabs the prey and bodily juices are then ingested (Pennak, 1978). Considering that previous AMNET samples showed nonimpairment, and the sediment sample collected was rich in taxa, it is likely that the missing *Hyalella azteca* fell prey to the nemertea present during the test.

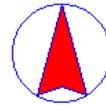
Survival results showed no significant differences between the control treatment and the Furnace Brook test. On days 4, 9, and 10, the Dissolved Oxygen fell below 40% saturation in 96H006b. The D.O. was 35%, 31%, and 38% respectively. Since mortalities were not substantial enough to show toxicity, the drop in D.O. probably did not adversely effect the test results. Although toxicity was not exhibited when compared to the control, additional toxicity testing should be performed on Furnace Brook with a more suitable reference sample, if future AMNET data continues to show severe impairment.

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- NONIMPAIRED AMHET SITE
- SEVERELY IMPAIRED AMHET SITE
- ⊕ HJPDES SITE
- ⊠ SOLID WASTE LANDFILL
- MAJOR ROADS
- STREAMS
- STREAM FLOW



**SEDIMENT
TOXICITY TEST
96H007a,
96H007b, 96H007c**



NJDEP
Division of Science & Research
Water Monitoring Management
Bureau of Water Monitoring

TABLE 1

MORTALITY DATA
(number surviving)

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	10	10	10	10	10	100
96H007b	10	10	8	8	6	84
96H007c	0	0	0	3	2	10

Statistical Analysis

Test Endpoint: Survival

Test Used: Wilcoxon Rank Sum Test

Results: 96H007c - significant difference from control

96H007d - no significant difference from reference station

*see appendix b for statistical printout

TABLE 2

WEIGHT DETERMINATION

Drying Oven Temperature: 105EC

Time/Date Start Drying: 1420 /11-1-96

Time/Date End Drying: 1620 /11-1-96

Analyst: T. Miller

REPLICATE.	WGT. OF BOAT (mg)	DRY WGT: BOAT + LARVAE (mg)	TOTAL WGT. OF LARVAE (mg)	NUMBER OF LARVAE	LARVAE AVG. DRY WGT. (mg)	GROUP AVG. (mg)
CONTROL A	20.61	22.00	1.39	10	0.139	0.119
B	15.49	16.43	0.94	10	0.094	
C	11.71	12.93	1.22	10	0.122	
D	15.59	16.66	1.07	10	0.107	
E	12.10	13.44	1.34	10	1.134	
95H007b A	16.15	18.76	2.61	10	0.261	0.229
B	15.10	17.33	2.23	10	0.223	
C	14.30	15.87	1.57	8	0.196	
D	17.42	19.29	1.87	8	0.234	
E	14.83	16.22	1.39	6	0.232	
95H007c A	---	---	---	0		0.316
B	---	---	---	0		
C	---	---	---	0		
D	14.31	15.14	0.83	3	0.277	
E	11.94	12.65	0.71	2	0.355	

Table 3**Test Chamber Chemical/Physical Parameters**

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.2	6.8	7.0	0.170	2.426
cond. Fmhos	141	130	136	3.557	2.609
D.O. mg/L	8.1	6.4	7.2	0.427	5.964

96H007b	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	6.8	6.5	6.6	0.129	1.938
cond. Fmhos	241	171	194	24.751	12.758
D.O. mg/L	5.1	2.7*	3.9	0.644	16.664

96H007c	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.0	6.6	6.8	0.138	2.025
cond. Fmhos	177	156	168	7.850	4.665
D.O. mg/L	6.8	4.2	5.0	0.825	16.620

* The D.O. fell below 40% saturation on days 4, 9, and 10 of the test.

APPENDIX A

AMNET DATA

Delaware Basin - Washington USGS Quadrangle
Station AN0042
Furnace Brook, Pequest Road, Oxford
October 8, 1992

Family	Number of Individuals	Family Tolerance Value (FTV)
Naididae	93	7
Asellidae	2	8
Gammaridae	1	4
BloodRedChironomidae	4	8

Statistical Analysis

Number of Taxa = 4

Total Number of Individuals = 100

% Contribution of Dominant Family = 93.00

Family Biotic Index = 7.03

Scraper/Filterer Collector Ratio = 0.00

Shredder/Total Ratio = 0.00

E+P+T* = 0 *(Ephemeroptera, Plecoptera and Trichoptera)

%EPT = 0.00

EPT/C* = 0.00 *(Chironomidae)

NJIS Rating = 0

Biological Condition = severely impaired

Deficiency(s) noted: paucity of clean water organisms

low diversity

Naididae overwhelmingly dominant

significant organic pollution

Observations

Streamwater: clear...Flow: moderate...Width/Depth(ft): 8/<1...

Substrate: rocks/gravel...Streambank Vegetation/Stability: good/
good...Canopy: open...Other: rural; below Oxford STP; lots of
algae and periphyton

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Delaware Basin - Blairstown USGS Quadrangle
 Station AN0045
 Beaver Brook, above Silver Lake, north of Hope
 September 16, 1992

Family	Number of Individuals	Family Tolerance Value (FTV)
Hydropsychidae	32	4
Philopotamidae	35	3
Limnephilidae	5	4
Chironomidae	6	6
Odontoceridae	3	0
Perlidae	4	1
Heptageniidae	6	4
Polycentropodidae	1	6
Elmidae	4	4
Peltoperlidae	2	1
Psephenidae	1	4
Asellidae	1	8

Statistical Analysis

Number of Taxa = 12
 Total Number of Individuals = 100
 % Contribution of Dominant Family = 35.00
 Family Biotic Index = 3.53
 Scraper/Filterer Collector Ratio = 0.13
 Shredder/Total Ratio = 0.10
 E+P+T* = 7 *(Ephemeroptera, Plecoptera and Trichoptera)
 %EPT = 86.00
 EPT/C* = 14.33 *(Chironomidae)
 NJIS Rating = 30
 Biological Condition = non-impaired
 Deficiency(s) noted: none

Observations

Streamwater: clear...Flow: moderate...Width/Depth(ft): 10/<1...
 Substrate: rocks/gravel...Streambank Vegetation/Stability: good/
 good...Canopy: partly open...Other: tree-lined; a darter

APPENDIX B
STATISTICAL DATA

Survival Proportions with Arc-Sine Square Root Transformation

Blank	AN0042	Blank Trans	AN0042 Trans
1	1	1.4127	1.4127
1	1	1.4127	1.4127
1	0.8	1.4127	1.1071
1	0.8	1.4127	1.1071
1	0.6	1.4127	0.8861

Shapiro-Wilks Test for Normality

Blank Trans	AN0042 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.1138	-0.4128				
1.4127	1.4127	1.4127		0.1138	-0.1918				
1.4127	1.1071	1.4127	1.2989	0.1138	-0.1918	0.3346	0.6552	0.842	Not Normal
1.4127	1.1071	1.4127		0.1138	0.1138				
1.4127	0.8861	1.4127		0.1138	0.1138				
		1.4127		0.1138	0.1138				
Mean	Mean	1.4127		0.1138	0.1138				
1.4127	1.1851	1.1071		-0.1918	0.1138				
		1.1071		-0.1918	0.1138				
		0.8861		-0.4128	0.1138				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0042	Critical (from Table K=1)	Result
1.4127	0.8861	10	1	0	1	19	No Significant Difference
1.4127	1.1071	9	2.5	0	2.5		
1.4127	1.1071	8	2.5	0	2.5		
1.4127	1.4127	7	7	0	7		
1.4127	1.4127	6	7	0	7		
1.4127	1.4127	5	7	7	0		
1.4127	1.4127	4	7	7	0		
1.1071	1.4127	3	7	7	0		
1.1071	1.4127	2	7	7	0		
0.8861	1.4127	1	7	7	0		
				Sum	Sum		
				35	20		

Survival Proportions with Arc-Sine Square Root Transformation

Blank	AN0045	Blank Trans	AN0045 Trans
1	0	1.4127	0.1581
1	0	1.4127	0.1581
1	0	1.4127	0.1581
1	0.3	1.4127	0.5796
1	0.2	1.4127	0.4636

Shapiro-Wilks Test for Normality

Blank Trans	AN0045 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	0.1581	1.4127		0.5546	-0.7				
1.4127	0.1581	1.4127		0.5546	-0.7				
1.4127	0.1581	1.4127	0.8581	0.5546	-0.7	3.2411	0.7421	0.842	Not Normal
1.4127	0.5796	1.4127		0.5546	-0.3945				
1.4127	0.4636	1.4127		0.5546	-0.2785				
		0.1581		-0.7	0.5546				
Mean	Mean	0.1581		-0.7	0.5546				
1.4127	0.3035	0.1581		-0.7	0.5546				
		0.5796		-0.2785	0.5546				
		0.4636		-0.3945	0.5546				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0045	Critical (from Table K=1)	Result
1.4127	0.1581	8	2	0	2	19	Significantly Different
1.4127	0.1581	7	2	0	2		
1.4127	0.1581	6	2	0	2		
1.4127	0.4636	10	4	0	4		
1.4127	0.5796	9	5	0	5		
0.1581	1.4127	5	8	8	0		
0.1581	1.4127	4	8	8	0		
0.1581	1.4127	3	8	8	0		
0.5796	1.4127	2	8	8	0		
0.4636	1.4127	1	8	8	0		
				Sum	Sum		
				40	15		